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# Effect of Adriamycin on DNA, RNA, and Protein Synthesis in Cell-free Systems and Intact Cells[1]

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## SUMMARY

The effect of adriamycin on DNA, RNA, and protein synthesis was investigated in cell-free systems and intact cells. In studies with purified mammalian cell enzymes, adriamycin produced a greater inhibition of DNA-dependent DNA polymerase than of RNA polymerase. The extent of inhibition of both these enzymes was decreased by increasing the concentration of the DNA template in the reaction mixture. In studies with isolated nuclei, adriamycin was also a more potent inhibitor of DNA synthesis than of RNA synthesis. However, with intact cells, adriamycin inhibited both DNA and RNA synthesis to about the same extent. The inhibition produced by adriamycin on RNA synthesis in intact cells was greater than that observed in the cell-free systems. Adriamycin inhibited protein synthesis in a cell-free system consisting of polyribosomes, transfer RNA, and enzymes but did not inhibit protein synthesis in intact cells. These differences in the pattern of inhibition may be due to biotransformation of the drug and/or preferential binding to chromosomal DNA in the intact cell.

## INTRODUCTION

Adriamycin, an anthracycline antibiotic, is an agent very potently cytotoxic to mammalian cells *in vitro* and *in vivo* (2, 12, 16). This antibiotic has been shown to be a very effective chemotherapeutic agent against tumors in experimental animals (17) and in humans (6). Cells in the S phase of the cell cycle appear to be most sensitive to the cytotoxic action of adriamycin (2, 12). Adriamycin inhibits both cellular DNA and RNA synthesis (12-14, 17, 18), presumably due to binding of this antibiotic to nucleic acids, as has been shown for the related antibiotic, daunorubicin (7). In studies with purified enzymes, adriamycin has been shown to inhibit the viral, bacterial, and mammalian cell DNA-dependent DNA polymerases (10, 18, 19) and bacterial RNA polymerase (19). From the published data on adriamycin it is not clear whether this antibiotic has a preferential specificity for the inhibition of DNA or RNA synthesis.

In this paper, in order further to understand the biochemical specificity of adriamycin, we have compared the effect of this antibiotic on DNA, RNA, and protein synthesis in various cell-free systems and in intact cells.

## MATERIALS AND METHODS

**Materials.** Adriamycin was obtained from the Drug Research and Development Branch of the National Cancer Institute, Bethesda, Md. Radioactive compounds and Aquasol scintillation fluid were obtained from New England Nuclear, Boston, Mass. Nucleotides were supplied by P-L Laboratories, Milwaukee, Wis. L-Amino acids were obtained from ICN Pharmaceuticals, Cleveland, Ohio. Phosphoenolpyruvate and pyruvate kinase were obtained from Calbiochem, La Jolla, Calif. The A($T_1$)Cl-3 hamster fibrosarcoma cells were kindly donated by Dr. W. F. Benedict (4). The cells were grown in suspension culture in Minimum Essential Medium $F_{14}$ containing 1 × nonessential amino acids (Grand Island Biological Co., Grand Island, N. Y.) and supplemented with 10% fetal calf serum (Flow Laboratories, Rockville, Md.). Minimal essential medium without leucine was obtained from Grand Island Biological Co. DNA-dependent DNA polymerase was prepared from calf thymus as described previously (14) except the DEAE-cellulose step was omitted; Fraction IX (Sepharose A) was used as the enzyme source. DNA-dependent RNA polymerase B was purified from calf thymus as described by Kedinger *et al.* (11); the DEAE-cellulose fraction was used as a source of enzyme. Denatured DNA was prepared by heating a solution of native calf thymus DNA (1.0 mg/ml in 10 mM NaCl containing 1.0 mM EDTA, pH 8.0) at 100° for 15 min and cooling immediately at 0°. The polyribosomes, tRNA, and enzymes involved in protein synthesis were prepared from reticulocytes of phenylhydrazine-treated rabbits by a modification (15) of the method of Gilbert and Anderson (8).

**Enzyme Assays.** The composition of the reaction mixtures for the DNA-dependent DNA polymerase and RNA polymerase assays is given in the tables. The enzymatic reaction was terminated by the addition of 5.0 ml of cold 5% trichloroacetic acid. The acid-insoluble material was collected on a Whatman GF/C glass fiber disc (2.4 cm diameter), washed twice with cold 5% trichloroacetic acid and ethanol, dried, placed in a toluene-based scintillation mixture, and assayed for radioactivity.

**Preparation of Isolated Nuclei.** A 200-ml suspension of A($T_1$)Cl-3 hamster fibrosarcoma cells (3 to 4 × $10^5$ cells/ml) was centrifuged at 900 × *g* for 4 min. The supernatant was discarded, and the cell pellet was suspended in 10 ml of

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tissue culture medium and centrifuged at 700 × g for 4 min. The cell pellet was suspended in 10 ml of 10 mM potassium phosphate, pH 7.5, containing 2 mM $MgCl_2$ (Buffer A) and was centrifuged at 700 × g for 4 min. The appropriate amount of Buffer A was added to the cell pellet to give a final cell concentration of $5 \times 10^7$ cells/ml. The cell suspension was left at 0–4° for 7 min, and then the cells were disrupted by 10 strokes in a Dounce homogenizer (5). The hypotonicity of the cell suspension was adjusted to isotonicity by addition of an equal volume of Buffer B (40 mM Tris-HCl, pH 7.5; 10 mM potassium phosphate buffer, pH 7.5; 160 mM KCl; 40 mM NaCl; 20 mM glucose; 2 mM 2-mercaptoethanol; and 0.1% Triton X-100). The suspension was centrifuged at 200 × g for 1 min, the supernatant was removed, and the nuclei were suspended in Buffer C (equal volumes each of Buffer A and Buffer B) at a concentration of $5 \times 10^7$ nuclei/ml. The nuclei were centrifuged at 200 × g for 1 min, suspended in Buffer C containing 30% glycerol (Buffer D), and stored at −20°. Under these storage conditions they maintained their biochemical activity for 1 week or more.

**Assay of DNA and RNA Synthesis in Isolated Nuclei.** The composition of the reaction mixture is given in the tables. The reaction was initiated by the addition of 50 μl of the reaction mixture to 100 μl of Buffer D containing $5 \times 10^6$ nuclei. The mixture was incubated for 30 min at 37°, and the reaction was stopped by the addition of cold 5% trichloroacetic acid. The acid-insoluble precipitate was washed 3 times with cold 5% trichloroacetic acid, hydrolyzed in 0.2 ml of 5% trichloroacetic acid at 100° for 45 min, and centrifuged, and the supernatant was assayed for radioactivity in 5 ml of Aquasol scintillation fluid.

**Assay of Protein Synthesis in Cell-free System.** The composition of the reaction mixture is given in the tables. The reaction was initiated by the addition of the enzyme fraction. After incubation at 37° for 20 min, the reaction was terminated by the addition of 2 ml of 10% trichloroacetic acid. The reaction tubes were heated at 90° for 10 min to hydrolyze any radioactive acylated tRNA, and the mixture was filtered on Whatman GF/C glass fiber disc (2.4 cm diameter). The discs were treated as described under "Enzyme Assays."

**Assay of Macromolecular Synthesis in Intact Cells.** $A(T_1)Cl$-3 hamster fibrosarcoma cells were grown routinely as a suspension in a Chemap Model E-1 Vibromixer. The cells (2 to $4 \times 10^5$ cells/ml) were centrifuged at 200 × g for 5 min and suspended in culture medium containing 5% dialyzed fetal calf serum at a cell density of $1.5 \times 10^5$ cells/ml. Two ml of the cell suspension were placed in plastic tubes containing 1 μCi of [$^3$H]thymidine, [$^3$H]uridine, or [$^3$H]leucine and adriamycin as indicated. For experiments with [$^3$H]leucine, culture medium without leucine was used. The tubes were flushed with air-5% $CO_2$, covered tightly, and incubated at 37° in a water bath shaker for 60 min. The cell suspension was poured onto Whatman GF/C glass fiber filters (2.4 cm diameter) that were previously washed with 0.9% NaCl solution and left wet. The cells on the filter were washed once with 5 ml cold 0.9% NaCl solution, twice with cold 5% trichloroacetic acid, and twice with absolute ethanol. The filters were then air-dried and assayed for radioactivity in scintillation fluid. The quenching of tritiated thymidine on the Whatman glass fiber discs in the presence of high concentrations of adriamycin (200 μg/ml) was negligible (less than 3%).

## RESULTS

The inhibitory effect of adriamycin on the DNA-dependent DNA polymerase reaction is shown in Table 1. When the concentration of denatured DNA in the reaction mixture was 100 μg/ml, the concentration of adriamycin that produced 50% inhibition was between 5.0 and 7.0 μg/ml. The reaction mixture for the DNA polymerase assay contained glucose in order to have a similar composition for the reaction mixture used for DNA synthesis in isolated nuclei (Table 4). According to the report of Bernard (5) there was greater DNA synthesis activity in isolated nuclei when glucose was included in the reaction mixture. The actual role of glucose in this system is not known.

The effect of adriamycin on the DNA-dependent RNA polymerase reaction is shown in Table 2. Adriamycin (about 25

Table 1

*Inhibition of DNA-dependent DNA polymerase reaction by adriamycin*

The reaction mixture (0.1 ml) contained 10 mM Tris-HCl, pH 7.5; 40 mM KCl; 10 mM NaCl; 6 mM $MgCl_2$; 5 mM potassium phosphate, pH 7.5; 2.5 mM ATP, 25 μM each dATP, dCTP, and dGTP; 222 pmoles [$^3$H]TTP ($3.9 \times 10^5$ cpm); 10 μg denatured DNA; 5 mM glucose; 0.5 mM 2-mercaptoethanol; 15% glycerol; 0.025% Triton X-100; 0.05 unit DNA polymerase; and the indicated concentrations of adriamycin. The mixture was incubated at 37° for 10 min and assayed as described under "Materials and Methods."

| Concentration of adriamycin (μg/ml) | [$^3$H]TTP incorporated (pmoles) | Inhibition (%) |
|---|---|---|
| 0 | 10.2 ± 0.5[a] | |
| 1.0 | 10.2 ± 0.6 | 0 |
| 2.5 | 7.8 ± 0.5 | 24 |
| 5.0 | 5.7 ± 0.7 | 44 |
| 7.0 | 3.7 ± 0.5 | 64 |
| 10.0 | 2.8 ± 0.5 | 73 |
| 20.0 | 0.2 ± 0.5 | 98 |

[a] Mean ± S.D.

Table 2

*Inhibition of DNA-dependent RNA polymerase reaction by adriamycin*

The reaction mixture (0.1 ml) contained 50 mM Tris-HCl, pH 8.0; 2.5 mM $MnCl_2$; 0.5 mM each of ATP, CTP, and GTP; 518 pmoles [$^3$H]UTP ($1.9 \times 10^5$ cpm); 20 μg denatured DNA; 0.1 unit RNA polymerase; and the indicated concentrations of adriamycin. The mixture was incubated at 37° for 30 min and assayed as described under "Materials and Methods."

| Concentration of adriamycin (μg/ml) | [$^3$H]UTP incorporated (pmoles) | Inhibition (%) |
|---|---|---|
| 0 | 32.8 ± 0.6[a] | |
| 2 | 34.8 ± 0.6 | 0 |
| 5 | 29.2 ± 0.7 | 11 |
| 10 | 24.2 ± 0.6 | 26 |
| 25 | 15.3 ± 0.9 | 53 |
| 30 | 10.7 ± 0.7 | 67 |
| 50 | 8.2 ± 0.5 | 75 |
| 100 | 2.1 ± 0.6 | 94 |

[a] Mean ± S.D.

μg/ml) produced 50% inhibition of the RNA polymerase reaction in the presence of denatured DNA (200 μg/ml).

The inhibition produced by a constant amount of adriamycin on the DNA and RNA polymerase reactions in the presence of different concentrations of denatured DNA is shown in Table 3. As the DNA concentration in the reaction mixture was increased, the inhibition of both the DNA and RNA polymerase reactions produced by a constant amount of adriamycin was reduced. For the DNA polymerase reaction, adriamycin (5 μg/ml) produced 80% inhibition in the presence of DNA (50 μg/ml) as comnpared to only 22% inhibition in the presence of DNA (200 μg/ml). At a concentration of 25 μg/ml, adriamycin produced 77% inhibition of the RNA polymerase reaction in the presence of DNA (50 μg/ml) as compared to 37% inhibition in the presence of DNA (200 μg/ml).

The inhibition of DNA synthesis by adriamycin in isolated nuclei from A($T_1$)Cl-3 hamster fibrosarcoma cells is shown in Table 4. DNA-dependent DNA polymerase was added to the reaction mixture in order to obtain measurable amounts of DNA synthesis (5). Under these conditions, adriamycin (4.0 μg/ml) produced 50% inhibition of DNA synthesis in the isolated nuclei.

The effect of adriamycin on RNA synthesis in isolated nuclei from the fibrosarcoma cells is shown in Table 5. The isolated nuclei produced measurable amounts of endogenous RNA synthesis and did not require the addition of RNA polymerase to the reaction mixture. Adriamycin at a concentration of 66.6 μg/ml produced about 50% inhibition of RNA synthesis in the isolated nuclei.

The effect of adriamycin on protein synthesis in the rabbit reticulocyte cell-free system is shown in Table 6. The cell-free system contained purified polyribosomes, tRNA, enzymes, and the cofactors required for protein synthesis. Adriamycin at concentrations of 10 and 100 μg/ml inhibited cell-free protein synthesis by 26 and 83%, respectively.

The effect of adriamycin on the incorporation of [$^3$H]thymidine, [$^3$H]uridine, or [$^3$H]leucine into the acid-insoluble fraction of fibrosarcoma cells is shown in Table 7. Adriamycin (5 μg/ml) inhibited both the incorporation of [$^3$H]thymidine into DNA and [$^3$H]uridine into RNA by about 40%. The effect of adriamycin on [$^3$H]uridine incorporation represents primarily the action of this antibiotic on RNA synthesis since, in the presence of cytosine arabinoside (10 μg/ml), which inhibits DNA synthesis by more than 90%, the same amount of inhibition of [$^3$H]uridine incorporation into RNA was produced by adriamycin (R. L. Momparler and F. Avila, unpublished observation). Adriamycin at concentrations from 5 to 50 μg/ml did not inhibit the incorporation of [$^3$H]leucine into protein but actually appeared to stimulate the uptake of this radioactive amino acid into protein.

Table 3

*Effect of amount of DNA on inhibition produced by adriamycin of DNA and RNA polymerase reactions*

The experimental conditions were the same as described in Tables 1 and 2 except the reaction mixture contained the indicated concentrations of adriamycin and denatured DNA.

| Concentration of DNA (μg/ml) | Inhibition of DNA synthesis by adriamycin (5 μg/ml) (%) | Inhibition of RNA synthesis by adriamycin (25 μg/ml) (%) |
|---|---|---|
| 25 | 90 | 83 |
| 50 | 80 | 77 |
| 100 | 58 | 58 |
| 200 | 22 | 37 |

Table 4

*Inhibition of DNA synthesis in isolated nuclei from hamster fibrosarcoma cells by adriamycin*

The reaction mixture contained 5 × 10$^6$ nuclei (0.1 ml Buffer D); 6 mM $MgCl_2$; 25 μM each of dATP, dCTP, and dGTP; 56 pmoles [$^3$H]TTP (1.0 × 10$^6$ cpm); 0.05 unit DNA polymerase; and the indicated concentrations of adriamycin in a total volume of 0.15 ml. The mixture was incubated at 37° for 30 min and assayed as described under "Materials and Methods."

| Concentration of adriamycin (μg/ml) | [$^3$H]TTP incorporated (pmoles) | Inhibition (%) |
|---|---|---|
| 0 | 0.36 | |
| 0.5 | 0.32 | 12 |
| 1.0 | 0.28 | 29 |
| 2.0 | 0.24 | 35 |
| 4.0 | 0.17 | 51 |

Table 5

*Inhibition of RNA synthesis in isolated nuclei from hamster fibrosarcoma cells by adriamycin*

The reaction mixture contained 5 × 10$^6$ nuclei (0.1 ml Buffer D); 6.7 mM $MgCl_2$; 333 μM each of ATP, CTP, and GTP; 500 pmoles [$^3$H]UTP (5.3 × 10$^5$ cpm); and the indicated concentrations of adriamycin in a total volume of 0.15 ml. The mixture was incubated at 37° for 30 min and assayed as described under "Materials and Methods."

| Concentration of adriamycin (μg/ml) | [$^3$H]UTP incorporated (pmoles) | Inhibition (%) |
|---|---|---|
| 0 | 2.04 | |
| 6.7 | 1.87 | 7 |
| 33.3 | 1.37 | 31 |
| 66.6 | 1.07 | 47 |
| 133.3 | 0.74 | 63 |

Table 6

*Inhibition of protein synthesis in reticulocyte cell-free system by adriamycin*

The reaction mixture (0.05 ml) contained 20 mM Tris-HCl, pH 7.5; 88 mM KCl; 4 mM $MgCl_2$; 1.0 mM ATP; 0.2 mM GTP, 3 mM potassium phosphoenolpyruvate; 0.08 mM each of L-amino acids (except L-leucine); 200 pmoles [$^3$H]leucine (4.8 × 10$^5$ cpm); 0.58 $A_{260}$ unit polyribosomes; 0.08 $A_{260}$ unit tRNA; 0.2 unit pyruvic kinase; 109 μg enzyme; and the indicated amounts of adriamycin. The mixture was incubated at 37° for 20 min and assayed as described under "Materials and Methods."

| Concentration of adriamycin (μg/ml) | L-[$^3$H]leucine incorporated (pmoles) | Inhibition (%) |
|---|---|---|
| 0 | 16.3 ± 0.3[a] | |
| 1 | 14.2 ± 0.6 | 13 |
| 10 | 12.0 ± 0.4 | 26 |
| 100 | 2.7 ± 0.3 | 83 |

[a] Mean ± S.D.

## DISCUSSION

Adriamycin, an effective antitumor agent (6, 9, 17), inhibits both DNA and RNA synthesis in mammalian cells (12–14, 17, 18), presumably by binding to nucleic acids (7). It is not clear whether this anthracycline antibiotic has a preferential biochemical specificity for the inhibition of DNA syn-

Table 7

*Effect of adriamycin on DNA, RNA, and protein synthesis in hamster fibrosarcoma cells*

The incubation mixture (2.0 ml) contained 3 × $10^6$ cells in culture medium containing 5% dialyzed serum; 1 μCi of [$^3$H]thymidine (20 Ci/mmole), [$^3$H]uridine (29.6 Ci/mmole), or [$^3$H]leucine (30.7 Ci/mmole) as indicated; and the indicated concentrations of adriamycin. The mixture was incubated at 37° for 60 min in a water bath shaker and assayed as described under "Materials and Methods."

| Concentration of adriamycin (μg/ml) | DNA synthesis | | RNA synthesis | | Protein synthesis | |
|---|---|---|---|---|---|---|
| | [$^3$H]Thymidine incorporated (cpm) | Inhibition (%) | [$^3$H]Uridine incorporated (cpm) | Inhibition (%) | [$^3$H]Leucine incorporated (cpm) | Inhibition (%) |
| 0 | 79,399 ± 1,702[a] | | 11,891 ± 833 | | 14,763 ± 464 | |
| 1 | 69,794 ± 1,134 | 12 | 10,489 ± 1,145 | 12 | 14,971 ± 109 | 0 |
| 5 | 47,077 ± 1,101 | 41 | 6,990 ± 221 | 41 | 16,304 ± 513 | 0 |
| 10 | 40,142 ± 1,518 | 49 | 4,951 ± 80 | 58 | 18,108 ± 85 | 0 |
| 20 | 22,573 ± 1,491 | 71 | 3,688 ± 117 | 69 | 18,629 ± 100 | 0 |
| 50 | 12,375 ± 1,800 | 84 | 1,586 ± 50 | 86 | 15,642 ± 224 | 0 |

[a] Mean ± S.D.

thesis to a greater extent than that of RNA synthesis because different results have been reported by various investigators. For example, the *in vitro* studies of Wang *et al.* (18) using L1210 cells and of Kim and Kim (12) using HeLa cells indicate that adriamycin is a more potent inhibitor of DNA synthesis than of RNA synthesis. In contrast to these observations, Meriwether and Bachur (13) found in their *in vitro* studies with L1210 cells that adriamycin inhibited DNA and RNA synthesis to the same extent. In addition, Zunino *et al.* (19) in studies with purified bacterial enzymes demonstrated that adriamycin inhibited RNA polymerase to a greater extent than did DNA polymerase. In order to clarify the biochemical specificity of adriamycin, we have compared the effect of this antibiotic on DNA, RNA, and protein synthesis in various mammalian cell-free systems and in intact cells.

In studies with purified mammalian cell enzymes, we found that adriamycin was a more potent inhibitor of DNA-dependent DNA polymerase than of RNA polymerase (Tables 1 and 2). Adriamycin (about 6 μg/ml) produced 50% inhibition of DNA polymerase as compared to the 25 μg/ml required to inhibit RNA polymerase by 50%. The reason for the different results published for the bacterial enzymes and the results reported here for the mammalian enzymes is not known. Presumably, for these mammalian enzymes, adriamycin has a greater affinity for the DNA polymerase binding site on the DNA template than for the binding site for RNA polymerase. Since the DNA template used in the polymerase assays was purified denatured DNA, the question arises whether adriamycin has the same binding specificity for chromosomal DNA of the cell. In our studies with isolated nuclei in which the chromosomal DNA is intact and no cytoplasmic enzymes are present to biotransform adriamycin, we again found that this antibiotic was a more potent inhibitor of DNA synthesis than of RNA synthesis (Tables 4 and 5). In the isolated nuclei the concentrations of adriamycin required to inhibit both DNA and RNA synthesis by 50% were about 4 and 67 μg/ml, respectively.

If adriamycin acts by binding to specific sites on the DNA template to inhibit both DNA and RNA synthesis, one would expect that the inhibition produced by this antibiotic when all the sites were occupied to approach 100%. Under these conditions the addition of more DNA template to the reaction mixture should provide free binding sites for the polymerases and thus reduce the amount of inhibition produced by adriamycin. This appears to be the case since we found that by increasing the concentration of DNA template in the reaction mixture, it was possible to reduce the inhibition produced by adriamycin on both the DNA and RNA polymerase reactions (Table 3). Similar results have been reported by Goodman *et al.* (10) and Zunino *et al.* (20) for the related antibiotic, daunorubicin.

In general, adriamycin does not appear to inhibit cellular protein synthesis, except at high concentrations and long exposure times (12, 18). In studies with the rabbit reticulocyte cell-free system containing polyribosomes, tRNA, cofactors, and enzymes, we found that adriamycin produced a significant inhibition of protein synthesis (Table 6). However, in our studies with intact cells this antibiotic did not inhibit protein synthesis (Table 7). Apparently, in the cell-free system adriamycin is capable of binding to some of the RNA molecules involved in protein synthesis. The preferential binding of adriamycin to chromosomal DNA in intact cells may leave only a few molecules of this antibiotic free to bind the RNA involved in protein synthesis.

Contrary to our results obtained in cell-free systems where adriamycin inhibited DNA synthesis to a greater extent than was RNA synthesis (Tables 1 to 5), we found that with intact cells this antibiotic inhibited both DNA and RNA synthesis to about the same extent (Table 7). It is possible that the purified enzymes used in our studies may not be the important enzymes with respect to the *in vivo* activity of adriamycin because there are several enzymes involved in both DNA and RNA replication in the cell. It is also possible that in intact cells adriamycin may be biotransformed to a metabolite that is a more potent inhibitor of RNA synthesis than the parent compound. For example, adriamycin is metabolized by the cytoplasmic enzyme, aldo-keto reductase, to adriamycinol, an active inhibitor of nucleic acid synthesis in cells (1). Studies on the comparative molecular pharmacology of antitumor antibiotics in cell-free systems and intact cells may provide a good method for determining whether biotransformation of these agents takes place at the cellular level and for determining whether various metabolites or new analogs of the parent compound have biological activity.

When correlating the *in vitro* data in this report with the *in vivo* effects of adriamycin, one should take into account

that much higher concentrations of this antibiotic were used *in vitro* than are observed in the body fluids after clinical administration of this drug. The antitumor activity of adriamycin is most likely due to inhibition of both RNA and DNA synthesis as a result of the binding of this antibiotic to cellular DNA. The inhibition of RNA synthesis by adriamycin may be the mechanism by which this antibiotic kills nonproliferating tumor cells (3). On the other hand, the increased sensitivity of tumor cells in the S phase of the cell cycle to the cytotoxic action of adriamycin (2, 12) may be due to the inhibition of both RNA and DNA synthesis.

## REFERENCES


1. Bachur, N. R. Adriamycin (NSC-123127) Pharmacology. Cancer Chemotherapy Rept., Part 3, *6:* 153–158, 1975.
2. Barranco, S. C., Gerner, E. W., Burk, K. H., and Humphrey, R. M. Survival and Cell Kinetics Effects of Adriamycin on Mammalian Cells. Cancer Res., *33:* 11–16, 1973.
3. Barranco, S. C., and Novak, J. K. Survival Response of Dividing and Nondividing Cells after Treatment with Hydroxyurea, Arabinosylcytosine, and Adriamycin. Cancer Res., *34:* 1616–1618, 1974.
4. Benedict, W. F., Rucker, N., Mark, C., and Kouri, R. E. Correlation between the Balance of Specific Chromosomes and the Expression of Malignancy in Hamster Cells. J. Natl. Cancer Inst., *54:* 157–162, 1975.
5. Bernard, O., Momparler, R. L., and Brent, T. P. Effect of DNA Polymerase on Nuclei from Different Phases of Cell Cycle. European J. Biochem., *49:* 565–571, 1974.
6. Bonadonna, G., Beretta, G., Tancini, G., Brambilla, C., Bajetta, E., DePalo, G. M., DeLana, M., Fossati-Bellani, F., Gasparini, M., Valagussa, P., and Veronesi, U. Adriamycin (NSC-0123127) Studies at the Institute Nazionale Tumori, Milan. Cancer Chemotherapy Rept., Part 3, *6:* 231–245, 1975.
7. Calendi, E., DiMarco, A., Regginani, M., Scarpinato, B., and Valentini, L. On Physico-Chemical Interactions between Daunomycin and Nucleic Acids. Biochim. Biophys. Acta, *103:* 25–49, 1965.
8. Gilbert, J. M., and Anderson, W. F. Cell-free Hemoglobin Synthesis II. Characteristics of the Transfer Ribonucleic Acid-dependent Assay System. J. Biol. Chem., *245:* 2342–2349, 1970.
9. Goldin, A., and Johnson, R. K. Experimental Tumor Activity of Adriamycin (NSC-123127). Cancer Chemotherapy Rept., Part 3, *6:* 137–145, 1975.
10. Goodman, M. F., Bessman, M. J., and Bachur, N. R. Adriamycin and Daunorubicin Inhibition of Mutant T4 DNA Polymerases. Proc. Natl. Acad. Sci. U. S., *71:* 1193–1196, 1974.
11. Kedinger, C., Gissinger, F., Gniazdowski, M., Mandel, J-L., and Chambon, P. Animal DNA-dependent RNA Polymerases. I. Large-Scale Solubilization and Separation of A and B Calf-Thymus RNA-Polymerase Activities. European J. Biochem., *28:* 269–276, 1972.
12. Kim, S. H., and Kim, J. H. Lethal Effect of Adriamycin on the Division Cycle of HeLa Cells. Cancer Res., *32:* 323–325, 1972.
13. Meriwether, W. D., and Bachur, N. R. Inhibition of DNA and RNA Metabolism by Daunorubicin and Adriamycin in L1210 Mouse Leukemia. Cancer Res., *32:* 1137–1142, 1972.
14. Momparler, R. L., Rossi, M., and Labitan, A. Partial Purification and Properties of Two Forms of Deoxyribonucleic Acid Polymerase from Calf Thymus. J. Biol. Chem., *248:* 285–293, 1973.
15. Momparler, R. L., Siegel, S., Avila, F., and Lee, T. Effect of tRNA from 5-Azacytidine Treated Hamster Fibrosarcoma Cells on *in Vitro* Protein Synthesis in a Cell-free System. Biochem. Pharmacol., *25:* 389–392, 1976.
16. Razek, A., Valeriote, F., and Vietti, T. Survival of Hematopoietic and Leukemic Colony-forming Cells *in Vivo* following Administration of Daunorubicin or Adriamycin. Cancer Res., *32:* 1496–1500, 1972.
17. Silvestrini, R., Lenaz, L., DiFronzo, G., and Sanfilippo, O. Correlations between Cytotoxicity, Biochemical Effects, Drug Levels, and Therapeutic Effectiveness of Daunomycin and Adriamycin on Sarcoma 180 Ascites in Mice. Cancer Res., *33:* 2954–2958, 1973.
18. Wang, J. J., Chervinsky, D. S., and Rosen, J. M. Comparative Biochemical Studies of Adriamycin and Daunorubicin in Leukemic Cells. Cancer Res., *32:* 511–515, 1972.
19. Zunino, F., Gambetta, R., and DiMarco, A. The Inhibition *in Vitro* of DNA Polymerase and RNA Polymerases by Daunomycin and Adriamycin. Biochem. Pharmacol., *24:* 309–311, 1975.
20. Zunino, F., Gambetta, R., DiMarco, A., Zacarra, A., and Luoni, G. A Comparison of the Effects of Daunomycin and Adriamycin on Various DNA Polymerases. Cancer Res., *34:* 754–760, 1975.